08-04-00 'PCT

533 Rec'd PCT/PTO 03 AUG 2000

☒

Form PTO-1390 U.S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE (Rev 1-98) TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		Attorney's Docket Number 16721-0023 U.S. Application No. (if known, see 37 CFR 1.5) 09/601665
International Application No. PCT/ FR99/00229	International Filing Date 03.02.99 (February 3, 1999)	Priority Date Claimed 03.02.98 (February 3, 1998)

OIPE AUG 03 2000 PATENT & TRADEMARK OFFICE

Title of Invention
MEDICINE FOR TREATING APOPTOSIS DYSFUNCTIONS CONTAINING OLIGOSACCHARIDES

Applicant(s) for DO/EO/US
YVIN, Jean-Claude; CRUZ, Florence; DESCAMPS, Valerie; RICHARD, Christophe; THIBAL, Vesna; ARRIGO, Patrick; CLOAREC, Bernard

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   - b. ☒ has been transmitted by the International Bureau.
   - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   - b. ☐ have been transmitted by the International Bureau.
   - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   - d. ☒ have not been made and will not be made.
8. ☒ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☒ A translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☐ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☐ Other items or information:

526 Rec'd PCT/PTO 03 AUG 2000

U.S. Application No. (if known see 37 CFR 1.5) 09/601665	International Application No. PCT/FR99/00229	Attorney's Docket Number 16721-0023

17. ☒ The following fees are submitted:

CALCULATIONS PTO USE ONLY

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $970.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $840.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO $760.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) $670.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) $96.00

ENTER APPROPRIATE BASIC FEE AMOUNT =				$ 840	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				$ ---	
Claims	Number Filed	Number Extra	Rate		
Total claims	10 - 20 =	0	x 18.00	$ ----	
Independent Claims	10 - 3 =	7	x 78.00	$ 546	
Multiple Dependent Claims (if applicable)			+ 260.00	$ ---	
TOTAL OF ABOVE CALCULATIONS =				$ 1,386	
Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28). -				$	
SUBTOTAL =				$ 1,386	
Processing fee of $130.00 for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$	
TOTAL NATIONAL FEE =				$ 1,386	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$	
TOTAL FEES ENCLOSED =				$ 1,386	
				Amount to be refunded:	$
				charged:	$

a. ☒ A check in the amount of $1,386 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 10-1215 in the amount of $__________ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Deposit Account No. 10-1215. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

Roger T. Frost, Reg. No. 22,176

SEND ALL CORRESPONDENCE TO:
Roger T. Frost, Esq.
JONES & ASKEW, LLP
2400 Monarch Tower, 3424 Peachtree Road, N.E.
Atlanta, Georgia 30326
Telephone: 404-949-2400

09/601665
8/PRTS
526 Rec'd PCT/PTO 03 AUG 2000

MEDICINE FOR TREATING APOPTOSIS DYSFUNCTIONS CONTAINING OLIGOSACCHARIDES

The subject of the invention is a medicine for treating apoptosis dysfunctions.

The term «apoptosis» is intended to refer to programmed cell death or cell suicide.

This death corresponds to a self-elimination of cells according to a defined program.

It reveals itself, initially, through bulges in the plasma membrane, these bulges being accompanied by a structural change in the membrane, and then through a loss of volume of the cell, which appears to contract and to collapse in on itself.

The nucleus condenses and the DNA is cleaved into small fragments (Raff, «*Nature*», 356, 397, 1992; Bortner et al., «*Trends in Cell. Biol.*» 5, 21, 1995).

In vivo, the cell undergoing apoptosis is recognized by macrophages which will phagocytose it and eliminate it without any inflammatory process.

Still in vivo, apoptosis is widely used by living organisms to control cell populations, in particular lymphocytes subsequent to their activation.

Moreover, during the development of organisms, apoptosis plays a fundamental role in the elimination of unnecessary embryonic tissues (lizard tail, rudiment of the genital organs of one sex or the other) and in the pattern of the organism (elimination of the interdigital webs between the future fingers etc.).

Some compounds which are present in living organisms specifically induce an apoptotic phenomenon. Thus, for example in mammals, the binding of the Fas ligand to the Fas membrane-bound receptor, which is also referred to as APO-1 or CD95, specifically induces an apoptosis; this apoptosis is used by the living organism

to control lymphocyte populations, in particlar T lymphocyte populations.

The abovementioned receptor and ligand represent an extremely advantageous physiological system which is involved in the specific elimination of cells which are no longer desired in the organism.

Mention may be made in particular of cell elimination during the maturation and activation of T lymphocytes. In fact, the Fas system, i.e. Fas ligand/Fas receptor, plays a fundamental role in the homeostasis of the immune system.

The Fas receptor is a member of a family of proteins which act as cell surface receptors and which also comprise the TNF (tumor necrosis factor) and NGF (nerve growth factor) receptors.

The Fas receptor is expressed in many cells; it is thought to accumulate in the Golgi apparatus.

The mechanism by which the Fas system induces cell death is unknown, but involves the activation of the proteases which are known under the designation ICE-like («interleukin-1 beta-converting enzyme») or caspases.

It may be noted that the Fas ligand can be secreted by cells in order to induce their own suicide; but given that this ligand is also found at the surface of activating cells, these cells will, as a result, induce the suicide of target cells by simple contact. Once it has been activated, the Fas receptor interacts with many intracellular proteins so as to transmit the apoptosis-triggering signal.

In vitro, other means exist for inducing apoptosis, for example by inhibiting the activity of certain kinases, and in particular kinase C; in this case, staurosporin may be used.

This product is very effective for inducing cell death by apoptosis.

It should, however, be noted that the signal transduction which is induced by staurosporin is different from that involving the Fas receptor.

However, while the means of activating apoptosis are different, the execution of the death program which is induced by these two modes of activation is equivalent and is characterized by an activation of the caspase cascade and a dysfunction of mitochondria, which releases compounds (for example the cytochrome C) which will promote the programmed destruction of the cell. This phenomenon is energy-dependent, but does not require the synthesis of new proteins. In fact, in a cell, everything is ready for it to carry out its own destruction.

In vivo, the regulation of the apoptotic phenomenon has a considerable importance.

Specifically, many pathologies are associated with its dysfunction.

Mention may be made, for example, of two cases of apoptosis dysfunction in which the apoptosis is modified via the Fas system: they are autoimmune diseases in which apoptosis is deficient and the destruction of HIV-1-infected CD4+ T lymphocytes in which apoptosis is too active.

In other cases such as the neuronal degeneration which is encountered, for example, in multiple sclerosis, apoptosis is activated via pathways which are as yet unknown.

Other pathologies exist in which apoptosis is deficient; in this respect, mention may be made of the accumulation of cancer cells in which apoptosis would appear to depend on the FAS system («*Green*», Science, vol. 278, 1246, 1997).

In light of the findings recalled above, the applicant company, to its credit, found that as a result of the availability of a medicine which is capable of

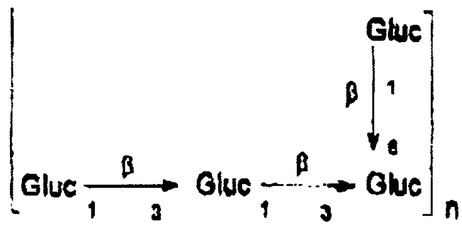
modifying apoptosis dysfunctions both from the point of view of an activation, in the case of the pathologies of the group comprising autoimmune diseases and the pathologies such as cancer, and from the point of view of its inhibition, in the case of the pathologies of the group comprising AIDS, it became possible to combat these diseases.

It also found, to its no less great credit, that some oligosaccharide and monosaccharide substances which optionally comprise, on at least some of their individual units, at least one substituent of the group comprising sulfate, methyl and acetyl groups, were capable of modifying apoptosis dysfunctions.

A subject of the invention is thus a medicine, characterized in that it comprises, as an active principle, an effective amount of at least one oligosaccharide substance which is capable of modifying apoptosis dysfunctions and which optionally comprises, on at least some of its individual units, at least one substituent of the group comprising sulfate, methyl and acetyl groups, said substance being chosen from the group comprising :

- the oligosaccharides which are derived, by enzymatic or chemical process, from the polymers of the group comprising (1→3)-β-glucans which optionally comprise (1→6)-β- branching,
- the oligosaccharides which are derived, by enzymatic or chemical process, from sulfated galactans, in particular carrageenans, agars and porphyrans.

According to one advantageous embodiment, the medicine in accordance with the invention comprises, as an active principle, an effective amount of at least one oligosaccharide which is capable of modifying apoptosis dysfunctions and which satisfies the formula :



(I)

in which n represents an integer from 1 to 50, preferably from 5 to 10, and in which the number of branches varies from 0 to 3 per repeat unit.

According to another advantageous embodiment, the medicine in accordance with the invention comprises, as an active principle, an effective amount of at least one repeat disaccharide which is capable of modifying apoptosis dysfunctions and which satisfies the formula:

$$\left[\mathrm{Gal}\xrightarrow[1\qquad 3]{\alpha}\mathrm{Gal}\xrightarrow[1\qquad 4]{\beta}\right]_n \mathrm{Gal} \qquad \text{(II)}$$

in which n represents an integer from 1 to 50, preferably from 1 to 20, at least some of the repeat disaccharides of formula (II) possibly comprising one or more sulfate groups.

According to another advantageous embodiment, the medicine in accordance with the invention comprises, as an active principle, an effective amount of the product which is capable of at least partially inhibiting apoptosis and which is obtained by hydrolysis from sodium iota-carrageenate, this product consisting of a mixture of oligo-iota-carrageenans which is referred to as T_9, which has a total saccharide content (determined according to Tillmans and Philippi) of 62%, and which has a distribution profile by size, which is estimated by electrophoresis on polyacrylamide gel according to

Zablakis and Perez, of

iota-neocarratetraose	(DP 2)	8-12%
iota-neocarrahexaose	(DP 3)	23-27%
iota-neocarraoctaose	(DP 4)	18-22%
iota-neocarradecaose	(DP 5)	13-17%
iota-neocarradodecaose	(DP 6)	8-12%
oligo-iota-carrageenan	(DP 7)	3- 7%
oligo-iota-carrageenans consisting of 8 to 15 repeat disaccharides	(DP 8-15)	13-17%.

The abovementioned methods are described in «*Botanica marina*», 33, 273-276 (1990) as regards Zablakis E. & Perez J., and in «*Biochem. Z.*», 215, 30-60 (1930), as regards Tillmans J. & Philippi K.

In order to prepare the product I_9, the following procedure may be carried out.

The iota-carrageenan is incubated in the presence of the partially purified enzyme iota-carrageenase at a temperature of 45 to 50°C, and then the hydrolysis products are ultrafiltered through a 10,000 Da membrane. The product I_9 is thus obtained.

More particularly, the iota-carrageenan polymer is hydrolyzed with a recombinant iota-carrageenase which is overexpressed in the strain *Escherichia coli.*

The preparation of the enzyme is carried out by dissolving the bacterial pellet (corresponding to 1 liter of culture) in 50 ml of 10 mM Tris pH 7.5, 100 mM NaCl, 5 mM $CaCl_2$ buffer so as to have a final concentration of 500 U/ml.

From a practical point of view, 100 g of iota-carrageenan substrate are dissolved in 20 l of distilled water while hot (80°C) so as to obtain at a concentration at 5 g/l, and then the pH is adjusted to 7.5 with ammonium carbonate.

To carry out the hydrolysis, the enzyme is added

to 50 U/g of polymer. The continuous ultrafiltration is begun after 30 minutes; it is a tangential ultrafiltration.

For this tangential ultrafiltration, a Pellicon machine comprising a PTGC 0.46 m^2 10,000 Da cassette from the company Millipore can be used; this machine is set to 2 bar at inlet and 0.5 bar at outlet.

The filtrate outlet is partially closed in order to maintain the filtration flow rate at 1 liter per hour.

The characteristics of the reaction chamber are chosen so as to allow the enzyme to be supplied with substrate until the 20 l of solution are used up and a fixed volume of 2 liters to be maintained.

18 l are obtained of an ultrafiltrate which is concentrated to 1 liter by rotary evaporation, and then the concentrate is lyophilized. The lyophilisate thus obtained contains the product I_9.

The oligocarrageenans of the I_9 fraction thus obtained were subjected to an additional fractionation by low pressure chromatography on a P6 Biogel column and then on a Sephadex G10 column.

The fractions identified above are thus obtained.

According to another advantageous embodiment, the medicine in accordance with the invention comprises, as an active principle, an effective amount of the product which is capable of at least partially inhibiting apoptosis and which consists of fraction DP7 of the product I_9.

According to another advantageous embodiment, the medicine in accordance with the invention comprises, as an active principle, an effective amount of the product which is capable of activating apoptosis dysfunctions, and which is obtained by acidic aqueous extraction from a brown alga named *Laminaria digitata*, this product consisting of a mixture of oligo-(1→3)-β-glucans which

are referred to as L_{11} and comprise from 1 to 50, preferably from 20 to 30, saccharide units, the product in question having the NMR spectrum shown in Figure 1.

It should be noted that the product L_{11} can also be obtained by aqueous extraction from brown algae in general, of which *Laminaria digitata* is a representative.

The preparation of the product L_{11} can be carried out as follows.

1 l of 0.3% sulfuric acid are gradually added to 300 g of fresh algae such as *Laminaria digitata* which are harvested in the month of August in fresh or dry form.

The procedure is carried out in a water bath at a temperature of approximately 70°C for 2 hours and 30 minutes with shaking.

This procedure is repeated twice.

The extract obtained is clarified by filtration through a filter with a porosity of 1.2 µm.

The liquid resulting from this filtration is subjected to a tangential ultrafiltration through a membrane with a porosity of 50,000 daltons.

The ultrafiltration is carried out while maintaining a pressure of 1 bar.

An ultrafiltrate whose pH is brought back to 5.5, and which has a volume of approximately 0.8 liters, is thus obtained. This ultrafiltrate is subjected to dialysis on a cellulose ester membrane which has a porosity equal to 500 daltons.

A dialysate is obtained which is concentrated to a volume of 100 ml by evaporating off at 80°C using a Rotovapor-type machine, and then lyophilized.

7 g of a cream-colored powder which constitutes the product L_{11} are obtained.

Analysis by ionic chromatography coupled with amperometry and using an ion-exchanging resin sold by the

company Dionex shows that the oligo-(1→3)-β-glucans which are constituents of the abovementioned powder in fact have 1 to 50, preferably from 20 to 30, saccharide units.

With the chromatographic conditions (so-called HPLC method, i.e. «High pressure liquid chromatography») being as follows:

Column	Carbopac PA1
Flow rate	1 ml/min
Detection	amperometric - gold electrode
Injection	50 µl
Elution gradient	50 mM sodium hydroxide-/500 mM sodium acetate - demineralized water
Analysis time	15 minutes
Retention time	≈ 9 - 10 minutes

the curve which is shown in Figure 16 was obtained which identifies the product L_{11}.

The examination of the ^{13}C NMR spectrum of the product L_{11}, which was carried out using an 80 mg/ml solution in D_2O and which is represented in Figure 1, shows a (1→3)-β-D-glucan backbone for which the resonances of the various carbons were able to be identified (they are assembled in Table A below) by comparison with the values in the literature [see Williams et al., 1992 *«Development of a water soluble, sulfated (1→3) β D-glucan biological response modifier derived from* ***Saccharomyces cerevisiae*****»**, Carbohydr. Res. 235: 247:251].

TABLE I

Chemical shifts (ppm) of the ^{13}C NMR spectrum of the sample L_{11}

(1→3)-β-D-glucan backbone	C1	102.76
	C2	73.41
	C3	84.94
	C4	68.45
	C5	75.89
	C6	61.06
D-mannitol residue	C6	63.42

The medicines in accordance with the invention which are defined above comprise the adjuvants of conventional formulation corresponding to their mode of administration and dose used.

A subject of the invention is also a method for preparing a medicine for treating apoptosis dysfunctions, characterized in that a pharmaceutical composition comprises at least one of the active principles identified above.

According to one advantageous embodiment, the abovementioned pharmaceutical composition is suitable for intravenous administration.

The invention is also directed toward the use, with a view to preparing a medicine for treating apoptosis dysfunctions, of the saccharide substances of the group comprising the oligosaccharides which are derived, by enzymatic or chemical process, from the polymers of the group comprising (1→3)-β-glucans which optionally comprise (1→6)-β- branchings, and the oligosaccharides which are derived, by enzymatic or chemical process, from

sulfated galactans, in particular carrageenans, agars and porphyrans.

More particularly, it is directed toward the use, with a view to preparing a medicine for treating apoptosis dysfunction, of the oligosaccharides of formula (I) and of those of formula (II).

Even more particularly, it is directed toward the use of the products which are referred to as I_9 and L_{11} and of fractions DP 2 and DP 7 of I_9 with a view to preparing medicines for treating apoptosis dysfunctions.

The invention will be even better understood with the aid of the further description which follows and of the examples which are in no way limiting, but which correspond to advantageous embodiments.

In the experiments which will be described below, work was carried out on cell cultures in which an apoptotic process was triggered using the Fas system or staurosporin, and then the modification effects possibly obtained with the products which constitute the active principle of the medicines in accordance with the invention were studied.

In the context of these experiments, on the one hand, the suitable amounts of active principle to obtain the desired effect of apoptosis modification and, on the other hand, the moment(s) at which it is suitable to administer the active principle or the medicine comprising it in order to obtain the desired modification effect were determined.

EXAMPLE 1

Work was carried out on a culture of murine fibroblasts which were genetically modified so as to constitutively express the human Fas receptor; the active principle tested was the product which is referred to as I_9.

In a prior experiment, it was shown that the murine fibroblasts are destroyed by apoptosis when they are placed in the presence either of the Fas ligand or of an agonist antibody which recognizes the Fas receptor and which, in the text hereinbelow, is referred to as FasAb.

In another prior experiment, it was determined that the product I_9 did not impair cell growth, that it was not toxic with respect to the murine fibroblasts at the concentrations used and that it could thus be added to a cell culture medium without creating problems.

The medium used for culturing the murine fibroblasts is that sold by the company Life Technologies under the name «Dulbecco's Modified Eagle Medium»; this medium is described in «*Virology*» 8, 396 (1959) by Dulbecco et al.

5% by volume of fetal calf serum was added to this medium.

This medium was then inoculated with murine fibroblasts in the presence of a sufficient amount of antibiotics to eliminate the possibilities of contamination; the fibroblast concentration of the culture medium was 10^5 cells per ml of medium.

Culturing was carried out in an incubator in which the temperature was kept at 37°C; the atmosphere filling the incubator contained 5% CO_2.

After 24 hours of incubation, either the Fas ligand or the FasAb was directly added to the medium.

The amount of FasAb added was 50 µg per ml of culture medium.

Under these conditions, approximately 70% of the cells of the culture are destroyed by apoptosis after approximately 24 hours of incubation.

This destruction is revealed by crystal violet staining of the surviving cells.

A certain number of assays were then carried out

which were intended to demonstrate the action of the active principle.

In these assays, on the one hand, the concentration at which the active principle is used in the culture medium and, on the other hand, the moment at which the active principle is added to this medium were varied so as to determine the optimal active principle concentrations, as well as the most appropriate moment(s) for introducing the active principle with respect to adding the FasAb.

The active principle concentrations were varied from 0.001 to 2 mg per ml.

Successive studies of the effect obtained by first of all adding the active principle before, then at the same time and finally after FasAb were carried out.

In a first assay, the active principle was added 24 hours before FasAb.

In the context of this assay, the effect obtained using successively 0, then 5, then 10, then 50, then 100 and finally 500 ng of FasAb per ml of culture and, in each case, active principle concentrations which were successively equal to 0.25, then 0.5 and finally 1 mg per ml of culture medium was scored, it being understood that the effect obtained in the absence of active principle, i.e. for a concentration of 0%, was also scored.

After 24 hours of incubation, the survival analysis is carried out.

The result of this analysis is indicated by the histogram in Figure 2.

The FasAb concentration of the medium expressed in ng/ml is shown on the x-axis of this histogram, and the level of survival expressed as % is shown on the y-axis.

For each of the FasAb concentrations, the level of survival for each of the four active principle concentrations, i.e. 0 mg/ml, 0.25 mg/ml, 0.5 mg/ml and

1 mg/ml, has been indicated with four rectangles which are parallel to the y-axis, the standard deviation being indicated each time with a segment which is situated on top of the corresponding rectangle, running parallel to the y-axis.

Each time, the rectangle corresponding to 0 mg/ml of active principle is the one which is located on the far left, the one corresponding to 0.25 mg/ml of active principle is located on its right, the one corresponding to 0.5 mg/ml is located to the right of the previous one, and so on.

Each time, the four rectangles are identified by characteristic hatchings, dots or shadings.

The examination of the results thus collated on the histogram in Figure 2 shows that, in the absence of active principle, the level of survival decreases as the FasAb concentration increases, and that this level of survival is noticeably improved by adding the active principle.

In a second assay, the FasAb and the active principle were added to the culture medium at the same time.

In this second assay, the effect obtained using successively the same FasAb and active principle concentrations as in the first assay was scored.

After 24 hours of incubation, the survival analysis is carried out.

The results recorded are collated on the histogram in Figure 3, which is prepared according to the same principles as those set out with respect to the one in Figure 2.

The examination of these results shows that the level of survival evolves in a way which is similar to that scored for the first assay.

In a third series of assays, the active principle

was added after FasAb, i.e. successively:

first of all, 1 hour after the FasAb,

then, 3 hours after the FasAb and,

finally, 6 hours after the FasAb,

always varying the FasAb concentration from 0 to 500 ng per ml of culture.

In the case of the addition of active principle carried out 1 hour after the FasAb,

- the results which were scored for I_9 concentrations of 0 mg/ml, of 0.005 mg/ml, of 0.01 mg/ml and finally of 0.05 mg/ml have been collated on the histogram in Figure 4,
- the results which were scored for I_9 concentrations of 0 mg/ml, of 0.1 mg/ml, of 0.25 mg/ml and finally of 0.5 mg/ml have been collated on the histogram in Figure 5, and
- the results which were scored for I_9 concentrations of 0 mg/ml, of 0.25 mg/ml, of 0.5 mg/ml and finally of 1 mg/ml have been collated on the histogram in Figure 6.

The histograms in Figures 4 to 6 are prepared according to the same principles as those set out with respect to the one in Figure 2.

In the case of the addition of active principle carried out 3 hours after the addition of FasAb, the results which were scored for I_9 concentrations of 0 mg/ml, of 0.25 mg/ml, of 0.5 mg/ml and of 1 mg/ml have been collated on the histogram in Figure 7.

In the case of the addition of active principle carried out 6 hours after the addition of FasAb, the results which were scored for I_9 concentrations of 0 mg/ml, of 0.25 mg/ml, of 0.5 mg/ml and of 1 mg/ml have been collated on the histogram in Figure 8.

The histograms in Figures 7 and 8 are prepared according to the same principles as those set out with

respect to the one in Figure 2.

The examination of all of the results which are collated on the histograms in Figures 4 to 8 shows that, in the case of adding the active principle after adding FasAb, the level of survival always increases; this effect has, however, a tendency to decrease when the time elapsed between the successive additions of FasAb and of active principle increases; moreover, it is sensitive to the active principle concentration when this concentration is lower than 0.25 mg/ml; no noticeable improvement is obtained for concentrations higher than 0.25 mg/ml.

In the experiment which has just been described, apoptosis was induced with the FasAb system.

Another experiment was carried out inducing apoptosis with the kinase inhibitor consisting of staurosporin.

It is recalled that staurosporin induces an apoptotic death in the case of most cells at doses varying from 0.5 to 5 µM.

In this experiment, the addition of staurosporin and of I_9 was simultaneous.

Two doses of I_9 were used, i.e. 0.2 mg/ml and 0.5 mg/ml.

Staurosporin was used in a proportion of 0.5 µM, then of 1 µM and finally of 1.5 µM.

The level of survival of the treated cells was determined in each case after 18 hours of incubation.

The results recorded appear on the histogram which is represented in Figure 9 and which shows the level of survival expressed as % as a function of the staurosporin concentration and for the doses of I_9 identified above.

A control experiment was carried out for a staurosporin concentration of 0 µM.

The examination of the results which appear in the histogram shows that the use of I_9 attenuates staurosporin-induced apoptosis.

From the two experiments which have just been decribed, it results that the medicine in accordance with the invention makes it possible to obtain a significant attenuation of apoptosis when this apoptosis is induced with various agents.

EXAMPLE 2

In this example, the cell culture studied was a culture of immortalized human cells consisting of T lymphocytes (Jurkat-type).

The culture medium used is that sold by the company Life Technologies under the name «RPMI 1640 Medium»; this medium is described by Moore et al. in the publication «A.M.A.» 199, 519 (1967).

10% by volume of fetal calf serum and antibiotics so as to eliminate the possibilities of contamination are added to this medium.

This medium is inoculated with an amount of 10^6 T lymphocytes per ml of medium.

The temperature of the incubation is 37°C and the atmosphere filling the incubator contains 5% CO_2.

After 24 hours of incubation, the FasAb and the active principle are added simultaneously.

The amount of FasAb (it is the one which is manufactured by the company Upstate Biotechnology and sold under the catalog No. 05-201 by the company Euromedex) added is 50 ng/ml of culture.

The active principle consists successively of the product I_9 and the product L_{11}.

In each case, the amounts used are 0.5 mg/ml.

The survival analysis is carried out 18 hours after the start of the experiment.

This survival analysis consists in passing a volume of culture containing 10^4 cells through a machine of the type which functions by flow cytometry, in this case the one sold by the company Beckton Dickinson under the name «FAC Scan cytometer».

This machine uses a probe which detects the presence of phosphatidylserine at the surface of cells; the presence of this product shows that the cells in question are apoptotic.

The results of this analysis appear in Figures 10 to 13 in each of which three polygons, A, B and C, respectively, are represented, the contours of which are defined so as to be representative of distinct cell populations; polygon A surrounds a set of living cells, polygon B a set of apoptotic cells and polygon C a set of dead cells.

The percentages of living cells and of apoptotic cells and the percentage of dead cells in the case of each of Figures 10 to 13, which will be commented upon below, have been collated in Table II hereafter.

TABLE II

	Living cells polygon A	Apoptotic cells polygon B	Dead cells polygon C
Control (Fig. 10)	97%	1%	1%
FasAb (Fig. 11)	77%	21%	1%
FasAb/Ia (Fig. 12)	90%	2%	6%
FasAb/L_{11} (Fig. 13)	74%	13%	9%

Figure 10 illustrates the survival analysis carried out on a sample of a culture medium to which neither FasAb nor active principle were added; it is a control; in this case, it is seen that there are

essentially only living cells, which are in polygon A (see line 1 of Table II).

Figure 11 illustrates the survival analysis carried out on a sample of a culture medium to which only FasAb was added; in this case, it is seen that polygon B contains 21% of apoptotic cells (see line 2 of Table II).

Figure 12 illustrates the survival analysis carried out on a sample of a culture medium to which FasAb and active principle I_9 (0.5 mg/ml) were simultaneously added; in this case, it is seen that polygon B contains practically no apoptotic cells (2%), polygon A containing many living cells and polygon C containing a certain concentration (6%) of dead cells (see line 3 of Table II).

Figure 13 illustrates the survival analysis carried out on a sample of a culture medium to which FasAb and active principle L_{11} (0.5 mg/ml) were simultaneously added; in this case, it is seen that polygon B contains a considerable amount (13%) of apoptotic cells and polygon C contains a not insignificant amount (9%) of dead cells (see line 4 of Table II).

The conclusions which can be drawn from the examination of Figures 10 to 13 and from the analysis of the percentage of the cells present in the various polygons mean, consequently, that the active principle I_9, in the context of this experiment, inhibits FasAb apoptosis (the percentage of apoptotic cells goes from 21% to 2%). However, a slight increase in the number of dead cells is noted (this number goes from 1% to 6%). If the number of living cells is considered, a protection of 13% is observed (the percentage goes from 77% to 90%).

With regard to the active principle L_{11}, an increase in the number of dead cells (the percentage goes from 1% to 9%) is observed with respect to the effect induced with FasAb only. The principle L_{11}, although not inducing a significant effect on the number of living

cells, is thus thought to act as a potentiator of cell death.

With the aim of optimizing the L_{11} action, the following experiments were carried out.

Using the same culture as above, there are administered:

➢ in a first experiment, 50 ng/ml of FasAb alone (of Euromedex origin identified above)

➢ in a second experiment, the same amount of the same FasAb simultaneously with 0.5 mg/ml of product L_{11} and

➢ in a third experiment, 0.5 mg/ml of the product L_{11} and then, 24 hours later, 50 ng/ml of the same FasAb.

The results obtained after 18 hours of incubation are as follows in comparison with what is observed on a control culture to which neither FasAb nor L_{11} have been added, the value taken into consideration being the number of living cells:

- in the case of addition of FasAb alone: the number of living cells decreases by 3.6%,
- in the case of the simultaneous addition of FasAb and of L_{11}, the number of living cells decreases by 6.4% and
- in the case of the deferred addition of FasAb and of L_{11}, the number of living cells decreases by 13.7%.

It ensues that L_{11} is much more active when it is administered before FasAb.

It is indicated that similar results were obtained when replacing the FasAb identified above with a FasAb of another origin, i.e. the one manufactured by the company Alexis Corporation (San Diego, USA) and sold by the company Coger SA (Paris).

It appears, from examining the results of the above experiments, that a medicine based on the product

I_{11} makes it possible to potentiate apoptosis; its use may thus be envisaged in the treatment of diseases of the autoimmune type and of cancer.

Other experiments made it possible to verify the effects produced by the administration of the product I_9, on the one hand, in lymphocyte cultures in which a low strength Fas apoptosis was induced and, on the other hand, in the case of lymphocyte cultures in which a high strength Fas apoptosis was induced.

The low strength Fas apoptosis can be induced using a FasAb originating from Euromedex; such an apoptosis can be of the order of 3 to 10%.

The high strength Fas apoptosis can be induced using a FasAb originating from Alexis Corporation; such an apoptosis can be of the order of 20 to 50%.

The experiments carried out using a dose of 0.2 mg/ml of the product I_9 are illustrated in Figure 14 which shows the effects recorded, i.e. either the stimulation or the inhibition of apoptosis (expressed as %) as a function of the strength of the apoptosis (as %) induced by FasAb alone (concentrations from 50 to 200 ng/ml).

Figure 14 shows that

- in the case of low strength apoptoses, of the order of 3 to 10%, which are indicated on the x-axis by the points a, b, c, d, e, f, g, h and i, the administration of I_9 causes a 20 to 40% potentiation of apoptosis with respect to the apoptosis induced by FasAb alone and
- in the case of high strength apoptoses, of the order of 20 to 50%, which are indicated on the x-axis by the points l, m, n, p and q, the administration of the same amount of I_9 causes a 5 to 20% inhibition of apoptosis with respect to the apoptosis produced by FasAb alone.

Knowing that it is possible to determine, in a given individual, the apoptosis induced in lymphocytes, it thus becomes possible to modify it according to the observed strength of said apoptosis in the direction of potentiation or in the direction of inhibition by administering, to this individual, a medicine based on I_9.

This medicine may, consequently, make it possible to correct apoptosis in the direction of potentiation when the individual is suffering from a disorder such as cancer or autoimmune disease which corresponds to weak apoptosis, and in the direction of an inhibition when the individual is suffering from a disorder such as immuno-deficiency syndrome which corresponds to strong apoptosis.

This being the case, it is specified that the Jurkat-type T lymphocytes present in the cultures which are subjected to the above experiments are cells in which the tumor suppressor gene p53 or p53 protein (Bing An et al., 1998, «Cell Death and Differentiation» 5, 1062-1075) is mutated and, as a result, inactive.

The effects observed with the products used in accordance with the invention, in particular with I_9 and L_{11}, are thus probably p53-independent; in other words, the products I_9 and L_{11} are capable of potentiating the stimulation or inhibition of apoptosis just by virtue of their presence and independently of the presence or absence of the active p53 gene.

Now, in most human cancers (see Hollstein et al., 1994, «Nucl. Acid Res.», 22, 3551), the p53 protein is mutated, and thus inactive; it ensues that the use of a medicine based on one of the products in accordance with the invention, and in particular on the products I_9 and L_{11} makes it possible to remedy p53 protein dysfunctions and to treat disorders such as cancer and autoimmune diseases.

The invention makes it possible, consequently, to treat the disorders in question in a way which is

fundamentally different and simpler than gene therapy, which is based on the principle aimed in particular at reintroducing a normal p53 gene into the genome of the mutated cells of a sick individual.

It is known that most cancer cells have lost their sensitivity to FasAb apoptosis and that most anticancer agents which are currently used act by activating the p53 gene which, itself, acts positively on the FasAb system. However, these systems no longer function if p53 is mutated (Müller et al. 1998, J. Exp. Med. 188, 2033-2043).

According to recent observations, the FasAb system is capital for the destruction of cancer cells either by the immune system or by the action of anticancer drugs; it ensues that the fact that the products used in accordance with the invention, and in particular I_9 and L_{11}, modify the apoptosis of said cancer cells in a p53-independent manner is of considerable importance and makes it possible to envisage the development of a novel generation of medicines in particular anticancer medicines.

EXAMPLE 3

The product I_9 is composed, as shown above, of a mixture of oligo-iota-carrageenans.

The effect induced by various constituents of this mixture, in particular of fractions DP 2, DP 3, DP 4, DP 5 and DP 7, was tested.

The polymer constituting the raw material of I_9 was also tested, as was the product named KIK (hexasaccharide of type kappa-iota-kappa).

For these experiments, the procedure was carried out as in Example 1, simultaneously introducing, on the one hand, 0.2 mg/ml of each of the abovementioned fractions of I_9 and, on the other hand, 100 ng/ml of FasAb

of Euromedex origin.

After 24 hours of incubation, the findings summarized below were made.

Fractions DP 2, DP 3, DP 4 and DP 5 are inactive and do not stimulate apoptosis induced by a low dose (100 ng/ml) of FasAb originating from Euromedex which induces, by itself, 6% of cell death after 18 hours of incubation.

Conversely, a very strong stimulatory effect is obtained for fraction DP 7 (50%) and with the product I_9 (70%), which shows that one of the active components of the I_9 mixture may consist of fraction DP 7; moreover, the product KIK has no activity.

Finally, it was found that the polymer constituting the raw material for the preparation of I_9 before cleavage with the enzyme iotase is, it too, active, and stimulates low strength Fas apoptosis. On the other hand, the enzyme iotase itself, used at 50 units, has no activity when it is incubated with the cells.

It ensues that the active principle I_9, which has no intrinsic apoptotic activity, induces a very strong stimulation in the case of low strength FasAb apoptosis.

EXAMPLE 4

A further experiment was carried out which confirms the results which are reflected by Figure 14.

In the case of the apoptotic phenomena induced by Fas, the first of the activated caspase cascade caspases is caspase 8.

Measurement was carried out of the activity of this capsase 8, which is a proteolytic enzyme, after lysis of Jurkat cells which had been treated or not with FasAb alone and with FasAb at the same time as with I_9.

To do this, measurement was carried out of

- the caspase 8 activity in a first fraction of a

culture of Jurkat cells in the absence of FasAb and of I_9; the percentage of living cells in the culture is 93%;

- the caspase 8 activity in a second fraction of the same culture, 18 hours after incubation with 500 ng/ml of FasAb originating from Euromedex (which, as indicated above, induces weak apoptosis, of the order of 13%); the percentage of living cells in the culture is 80%;
- the caspase 8 activity in a third fraction of the same culture, 18 hours after incubation with 500 ng/ml of FasAb originating from Euromedex and of 0.2 mg/ml of I_9; the percentage of living cells in the culture is 50%;
- the caspase 8 activity in a fourth fraction of the same culture, 18 hours after incubation with 25 ng/ml of FasAb originating from Alexis (which, as indicated above, induces strong apoptosis, of the order of 40%); the percentage of living cells in the culture is 53%;
- the caspase 8 activity in a fifth fraction of the same culture, 18 hours after incubation with 25 ng/ml of FasAb originating from Alexis and of 0.2 mg/ml of I_9; the percentage of living cells in the culture is 59%;

The measurement of caspase activity was carried out each time using the kit sold by the company Ozyme under the name «Apo Alert Flice Fluor» No. K2028-2.

The results of these measurements are collated in the histogram in Figure 15.

The examination of this histogram shows that

- the caspase 8 activity is weakly stimulated (factor in the vicinity of 1.37) by Euromedex FasAb alone, the simultaneous administration of I_9 inducing a strong stimulation (factor in the vicinity of 3.93);

- the caspase 8 activity is strongly stimulated (factor in the vicinity of 4.96) by Alexis FasAb alone, this strong stimulation being decreased (factor in the vicinity of 4.05) when I_9 is introduced simultaneously with Alexis FasAb.

Consequently, a clear correlation exists between apoptosis and caspase 8 activity.

The product I_9 thus represents a product which is capable of modifying caspase 8 activity.

The fact that it is shown that this enzyme is essential in the biochemical pathway of apoptosis induced by Fas (Juo et al., Curr. Biol. 8, 1001-1008, 1998), makes it possible to envisage novel medicines based on oligosaccharides for targeting enzymes such as caspase.

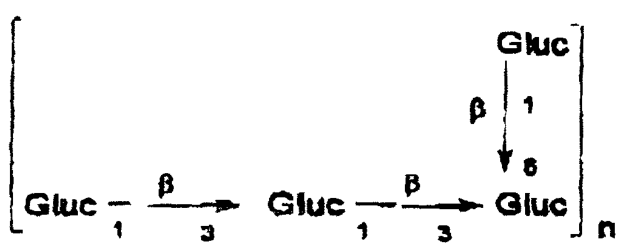
These products thus represent an alternative to a therapy based on peptides which bear the sequence recognized by caspase 8 (caspase 8 «suicide substrates»).

CLAIMS

1. Medicine, characterized in that it comprises, as an active principle, an effective amount of at least one oligosaccharide substance which is capable of modifying apoptosis dysfunctions and which optionally comprises, on at least some of its individual units, at least one substituent of the group comprising sulfate, methyl and acetyl groups, said substance being chosen from the group comprising :

- the oligosaccharides which are derived, by enzymatic or chemical process, from the polymers of the group comprising $(1\rightarrow3)$-β-glucans which optionally comprise $(1\rightarrow6)$-β- branching, and
- the oligosaccharides which are derived, by enzymatic or chemical process, from sulfated galactans, in particular carrageenans, agars and porphyrans.

2. Medicine, characterized in that it comprises, as an active principle, an effective amount of at least one oligosaccharide which is capable of modifying apoptosis dysfunctions and which satisfies the formula:



(I)

in which n represents an integer from 1 to 50, preferably from 5 to 10, and in which the number of branches varies from 0 to 3 per repeat unit.

3. Medicine, characterized in that it comprises, as an active principle, an effective amount of at least one repeat disaccharide which is capable of modifying apoptosis dysfunctions and which satisfies the formula :

$$\left[Gal \xrightarrow[1 \quad 3]{\alpha} Gal \xrightarrow[1 \quad 4]{\beta} \right]_n Gal \qquad (II)$$

in which n represents an integer from 1 to 50, preferably from 1 to 20, at least some of the repeat disaccharides of formula (II) possibly comprising one or more sulfate groups.

4. Medicine, characterized in that it comprises, as an active principle, an effective amount of the product which is capable of at least partially inhibiting apoptosis and which is obtained by hydrolysis from sodium iota-carrageenate, this product consisting of a mixture of oligo-iota-carrageenans which is referred to as I_9, which has a total saccharide content (determined according to Tillmans and Philippi) of 62%, and which has a distribution profile by size, which is estimated by electrophoresis on polyacrylamide gel according to Zablakis and Perez, of

iota-neocarratetraose	(DP 2)	8-12%
iota-neocarrahexaose	(DP 3)	23-27%
iota-neocarraoctaose	(DP 4)	18-22%
iota-neocarradecaose	(DP 5)	13-17%
iota-neocarradodecaose	(DP 6)	8-12%
oligo-iota-carrageenan	(DP 7)	3- 7%
oligo-iota-carrageenans consisting of 8 to 15 repeat disaccharides	(DP 8-15)	13-17%.

5. Medicine, characterized in that it comprises as an active principle, an effective amount of the product which is capable of activating apoptosis dysfunctions, and which is obtained by acidic aqueous extraction from brown algae and more particularly from a brown alga named *Laminaria digitata*, this product consisting of a mixture of oligo-(1→3)-β-glucans which are referred to as L_{11} and

comprise from 1 to 50, preferably from 20 to 30, saccharide units, the product in question having the NMR spectrum shown in Figure 1.

6. Medicine, characterized in that it comprises, as an active principle, an effective amount of the product which is capable of activating apoptosis dysfunctions and which consists of fraction DP 7 of the product I_9.

7. Method for preparing a medicine for treating apoptosis dysfunction, characterized in that a pharmaceutical composition comprises at least one of the active principles of the medicine according to at least one of Claims 1 to 6.

8. Use, with a view to preparing a medicine for treating apoptosis dysfunctions, of at least one of the oligosaccharide substances which optionally comprise, on at least some of their individual units, at least one substituent of the group comprising sulfate, methyl and acetyl groups, said substances which are capable of modifying apoptosis dysfunctions being chosen from the group comprising

- the oligosaccharides which are derived, by enzymatic or chemical process, from the polymers of the group comprising $(1\rightarrow3)$-β-glucans which optionally comprise $(1\rightarrow6)$-β- branching, and
- the oligosaccharides which are derived, by enzymatic or chemical process, from sulfated galactans, in particular carrageenans, agars and porphyrans.

9. Use, with a view to preparing a medicine for treating apoptosis dysfunctions, of the oligosaccharides of formula (I) and of those of formula (II).

10. Use of the products which are referred to as I_9 and L_{11} and of the product constituting fraction DP 7 of the product I_9 with a view to preparing medicines for treating apoptosis dysfunctions.

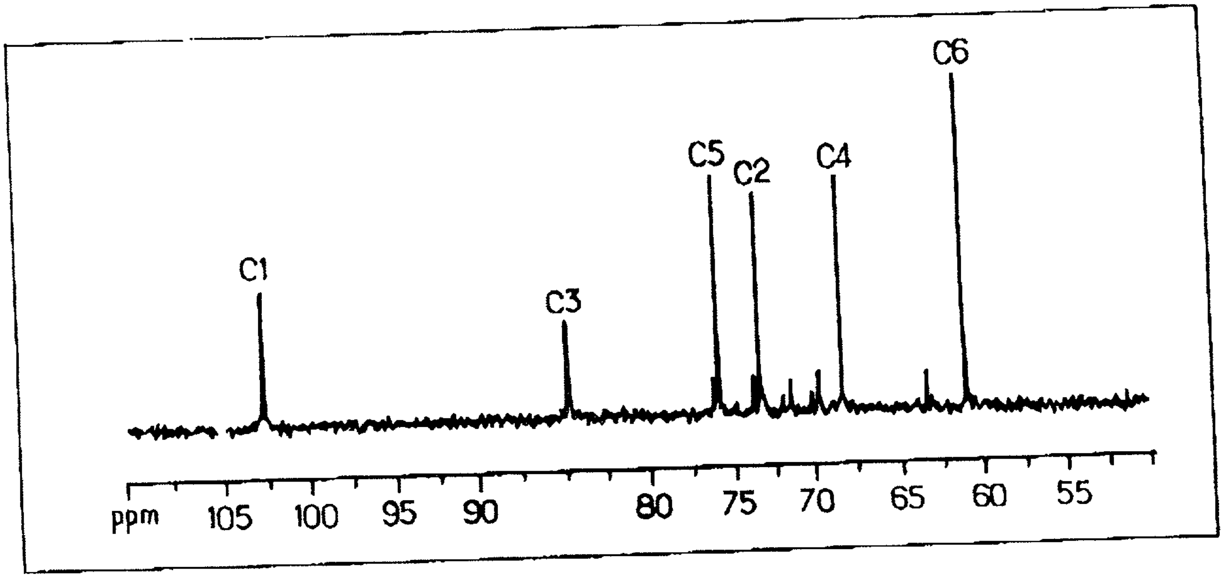

C1
C3
C5
C2
C4
C6
ppm
105
100
95
90
80
75
70
65
60
55

FIG.1.

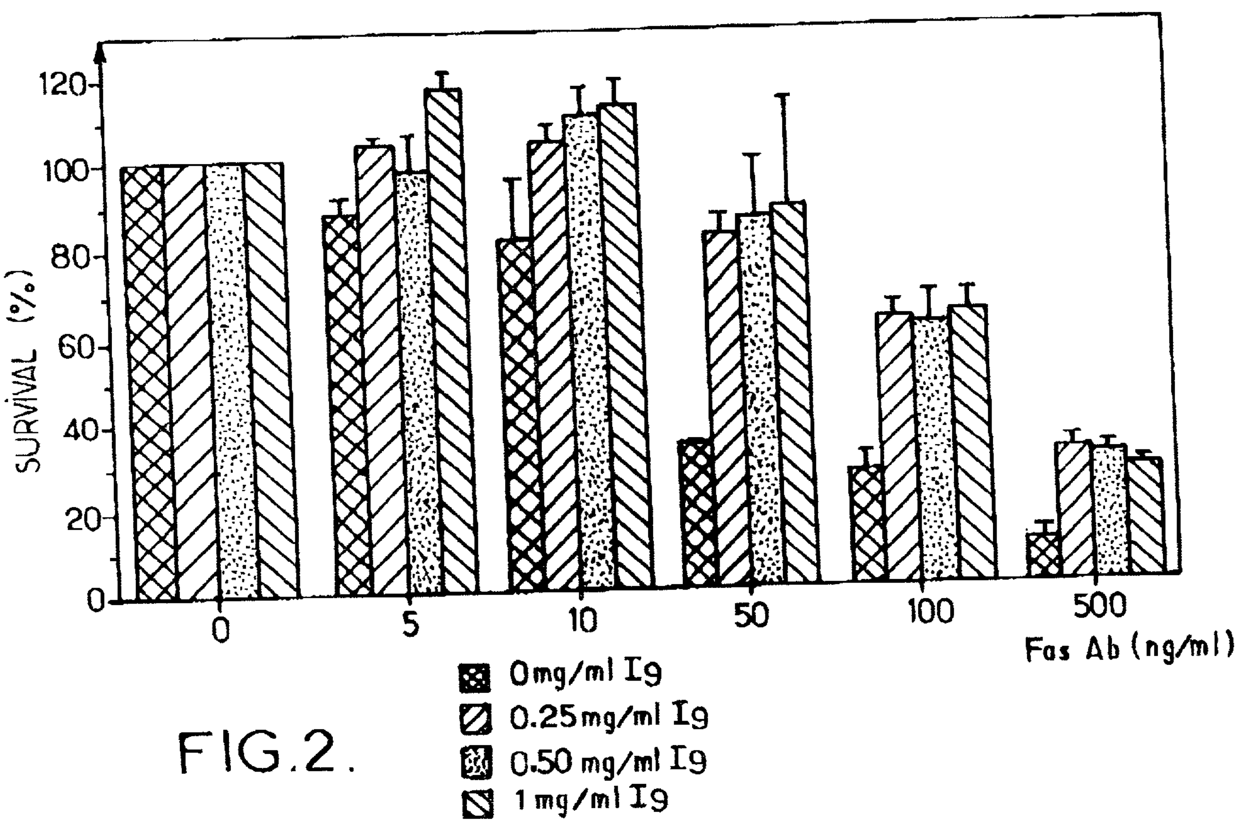
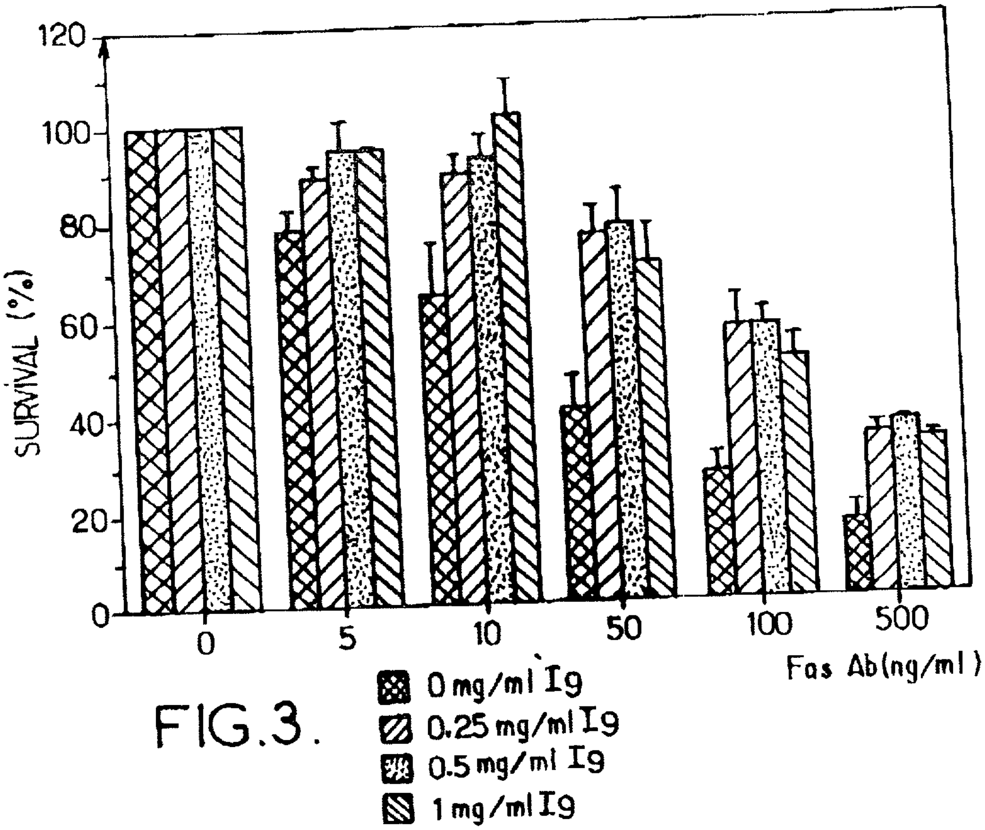
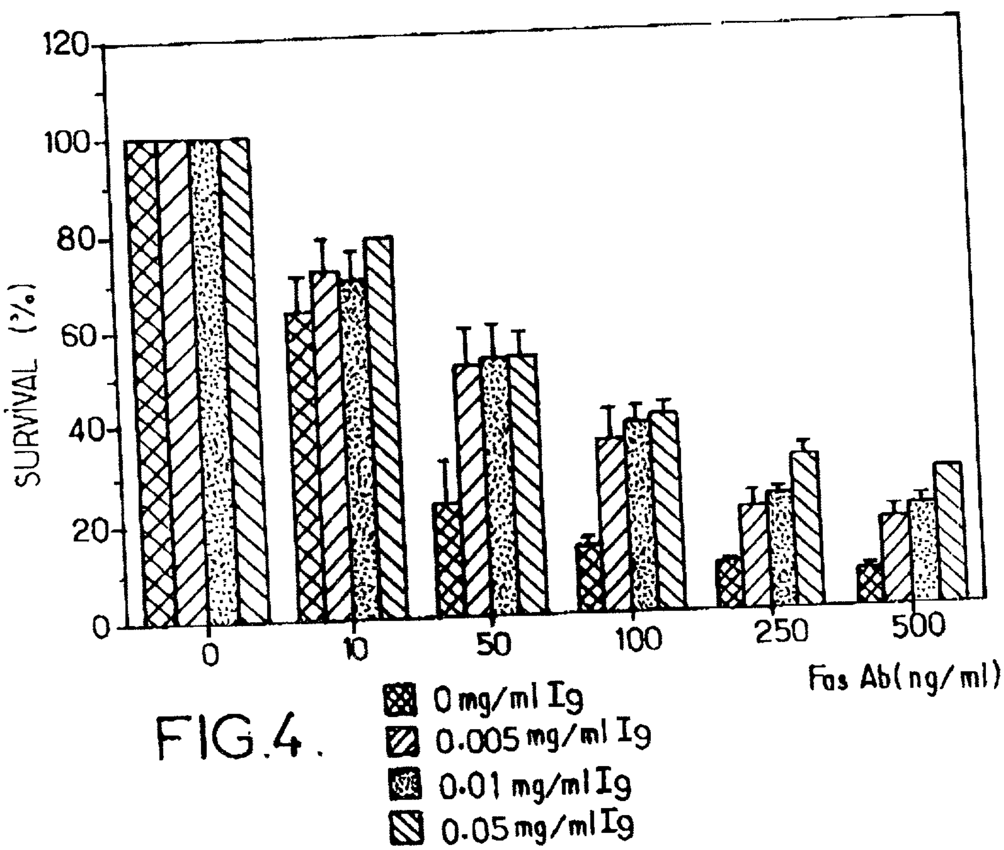

120
100
80
60
40
20
0
SURVIVAL (%)
0
5
10
50
100
500
Fas Ab (ng/ml)
0mg/ml I9
0.25mg/ml I9
0.50 mg/ml I9
1 mg/ml I9

FIG.2.



120
100
80
60
40
20
0
SURVIVAL (%)
0
5
10
50
100
500
Fas Ab(ng/ml)
0 mg/ml Ig
0.25 mg/ml Ig
0.5 mg/ml Ig
1 mg/ml Ig

FIG.3.



120
100
80
60
40
20
0
SURVIVAL (%)
0
10
50
100
250
500
Fas Ab(ng/ml)
0 mg/ml Ig
0.005 mg/ml Ig
0.01 mg/ml Ig
0.05 mg/ml Ig

FIG.4.

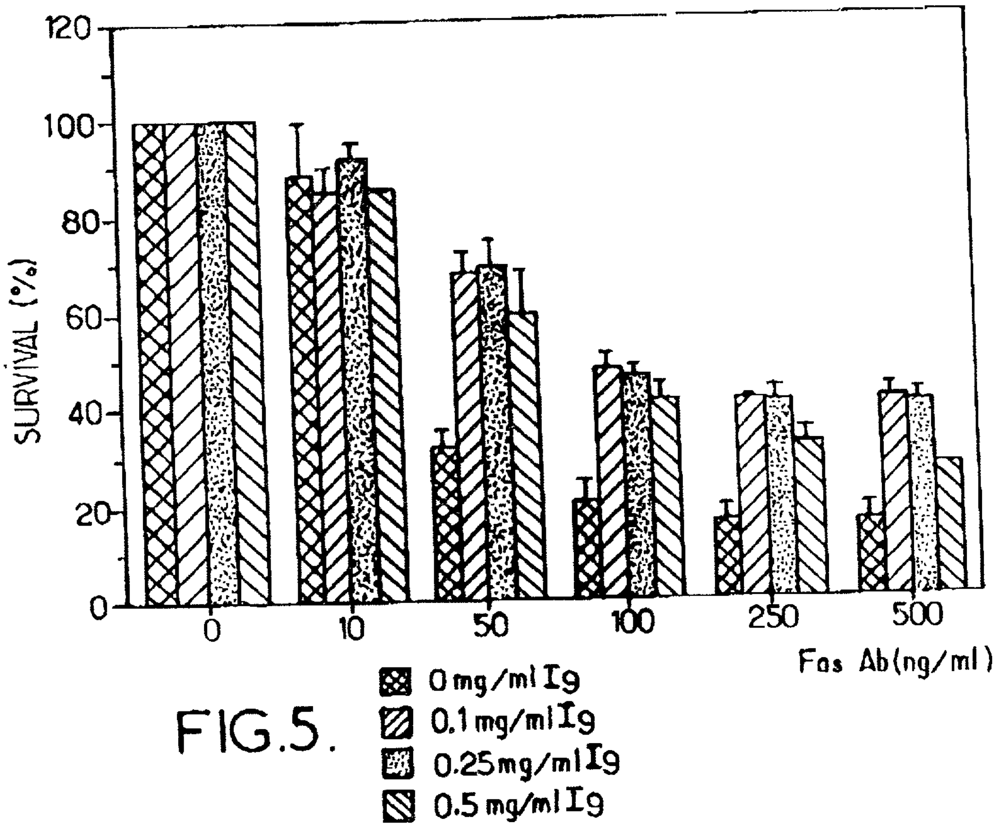

120
100
80
60
40
20
0
SURVIVAL (%)
0
10
50
100
250
500
Fas Ab(ng/ml)
0 mg/ml Ig
0.1 mg/ml Ig
0.25 mg/ml Ig
0.5 mg/ml Ig

FIG.5.

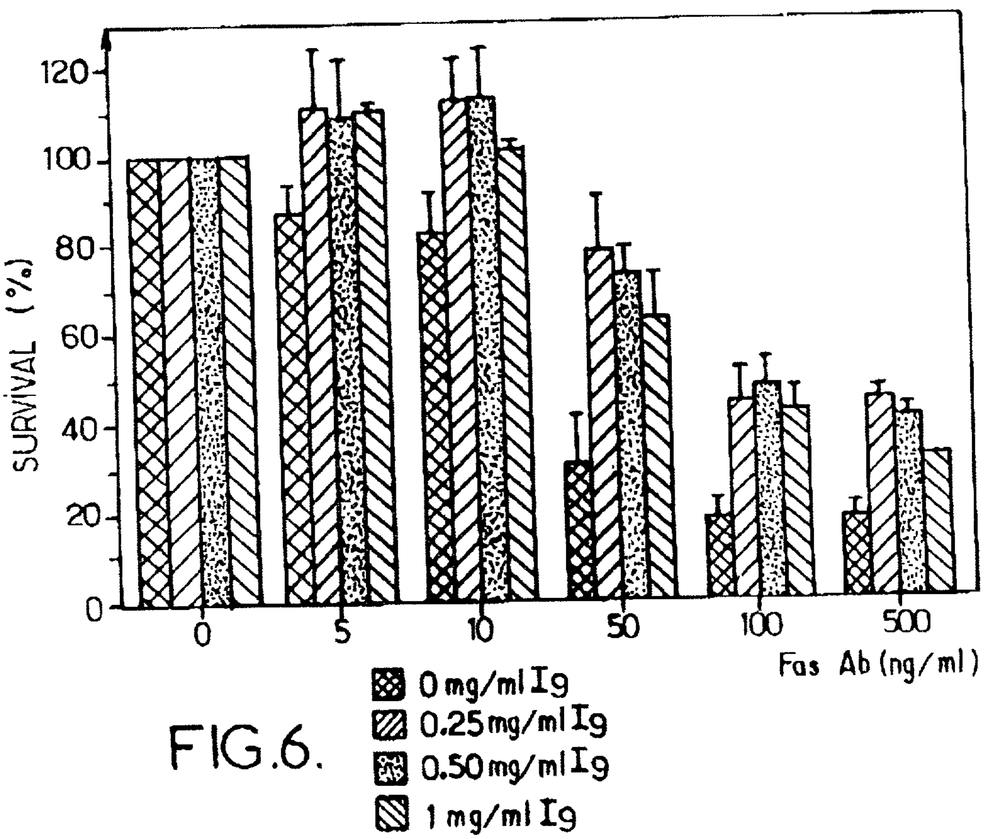

120
100
80
60
40
20
0
SURVIVAL (%)
0
5
10
50
100
500
Fas Ab (ng/ml)
0 mg/ml Ig
0.25 mg/ml Ig
0.50 mg/ml Ig
1 mg/ml Ig

FIG.6.

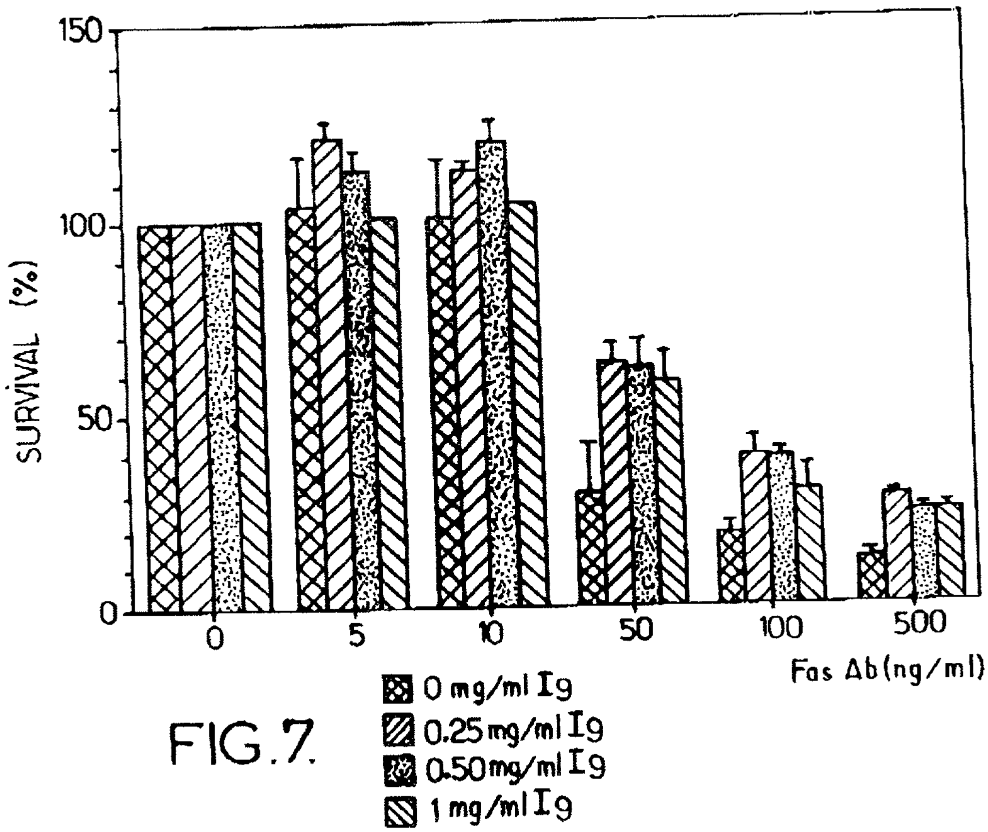

150
100
50
0
SURVIVAL (%)
0
5
10
50
100
500
Fas Ab (ng/ml)
0 mg/ml Ig
0.25 mg/ml Ig
0.50 mg/ml Ig
1 mg/ml Ig

FIG.7.

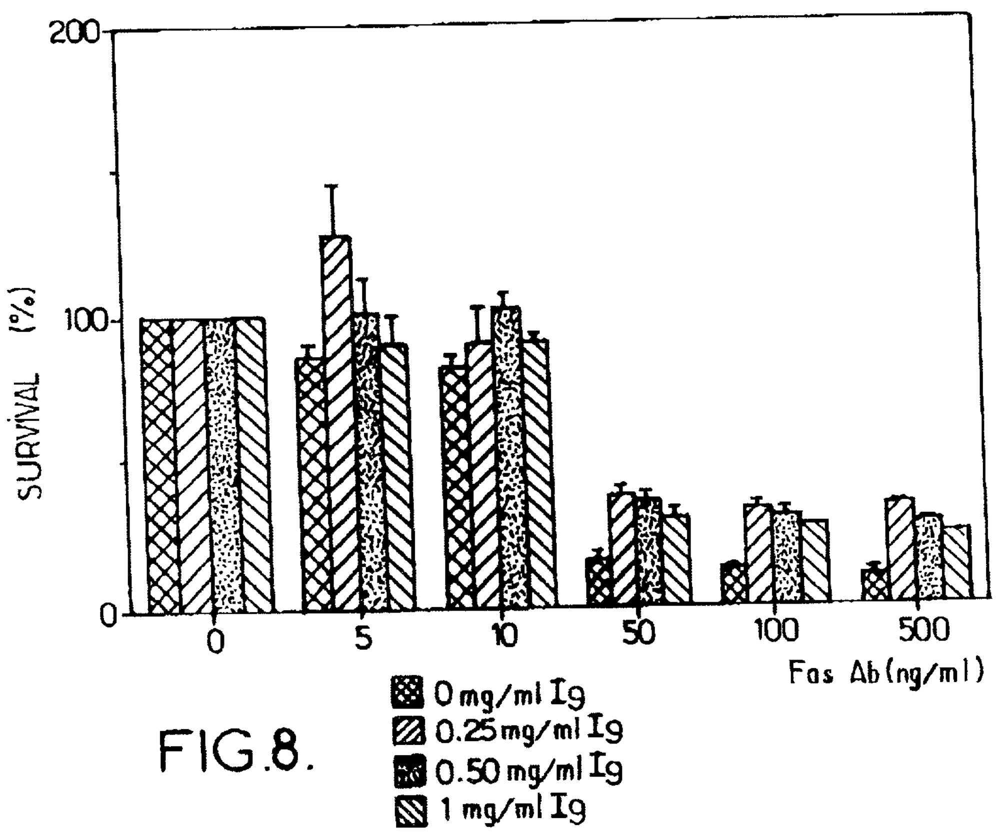

200
100
0
SURVIVAL (%)
0
5
10
50
100
500
Fas Ab (ng/ml)
0 mg/ml Ig
0.25 mg/ml Ig
0.50 mg/ml Ig
1 mg/ml Ig

FIG.8.

09/601665

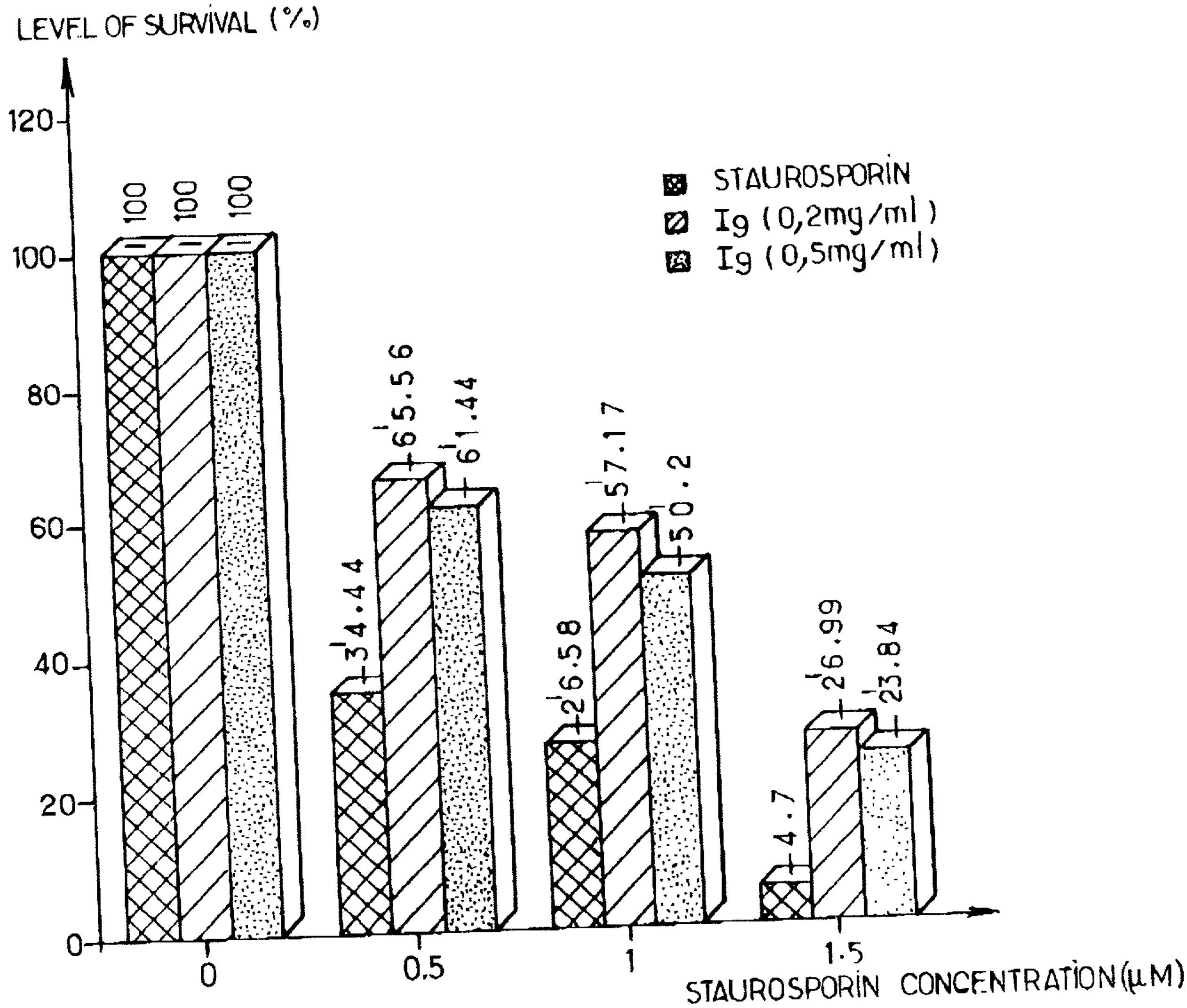


FIG.9.

FIG.10.

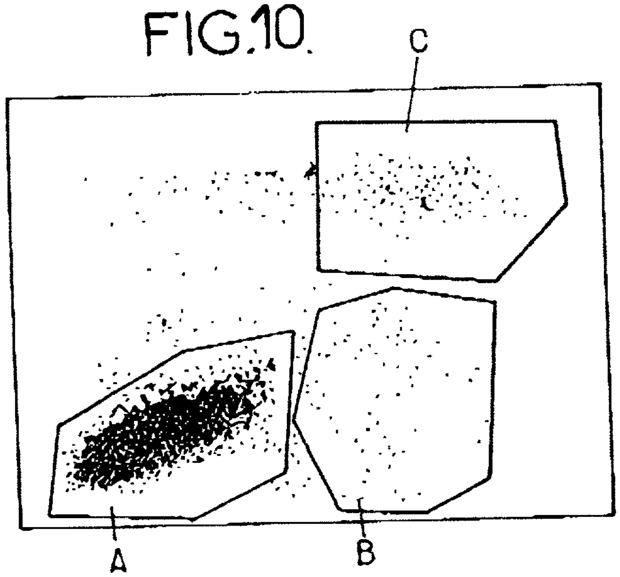


FIG.11.

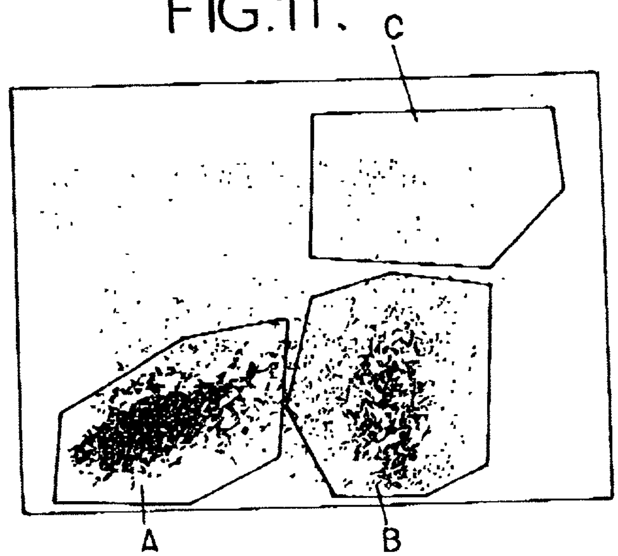


FIG.12.

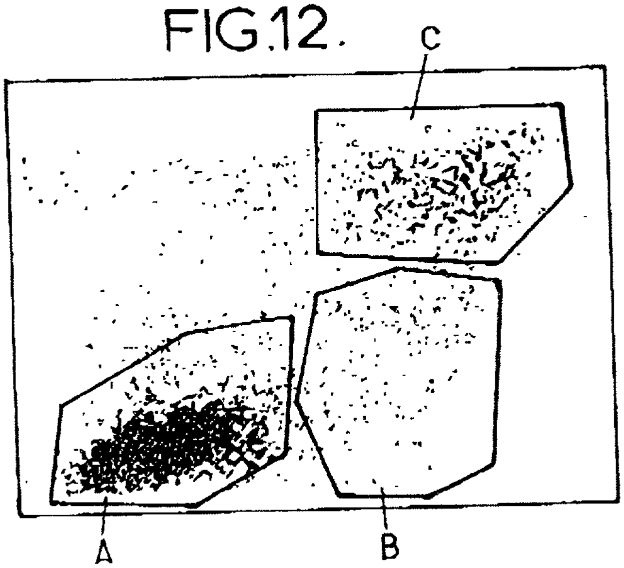
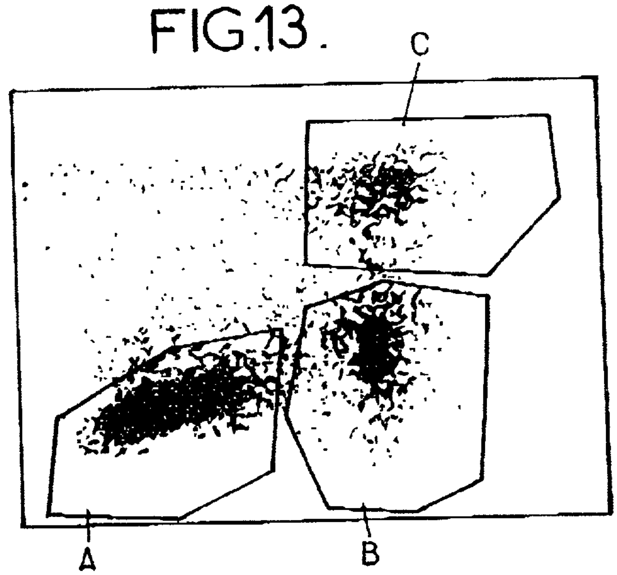
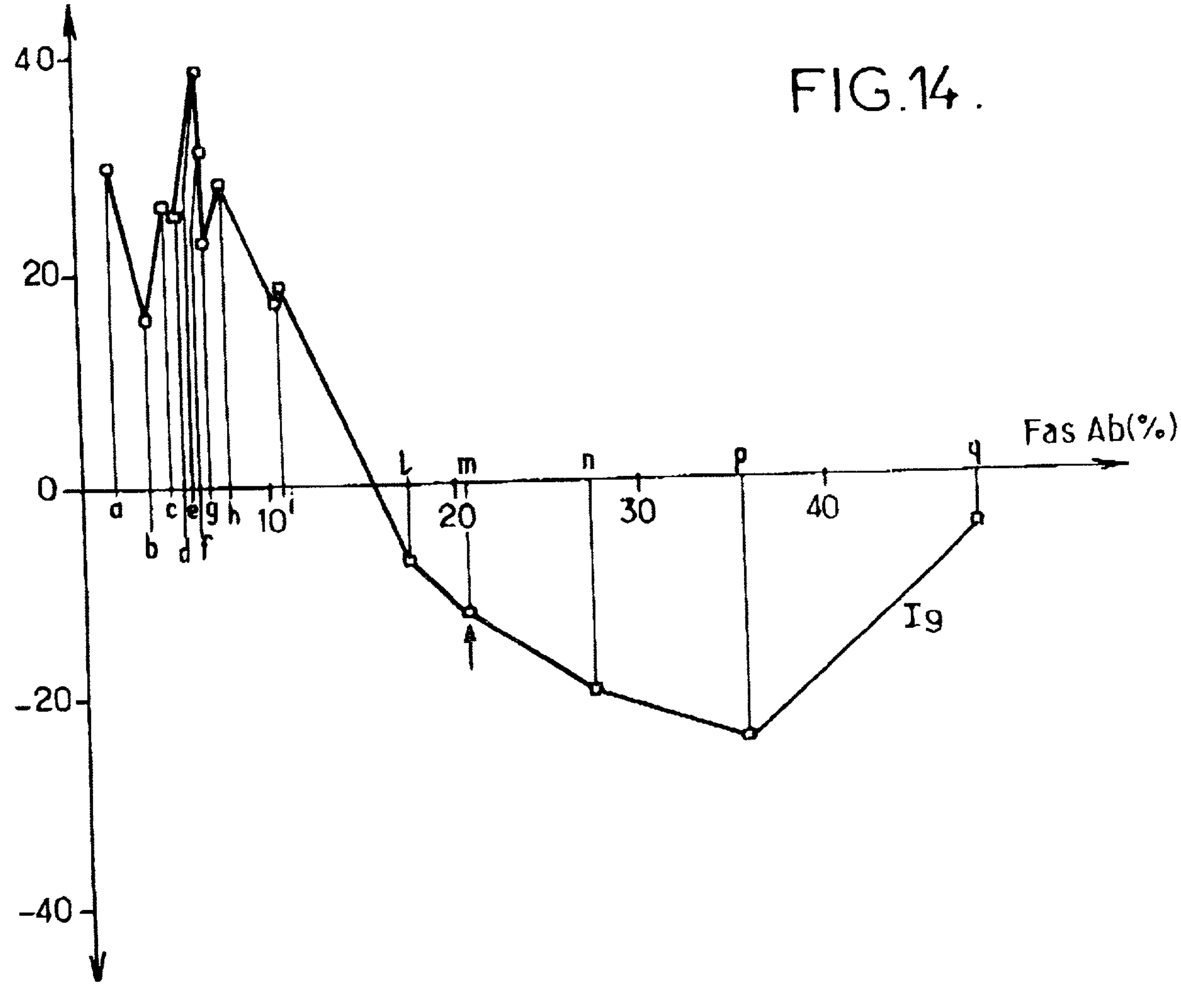


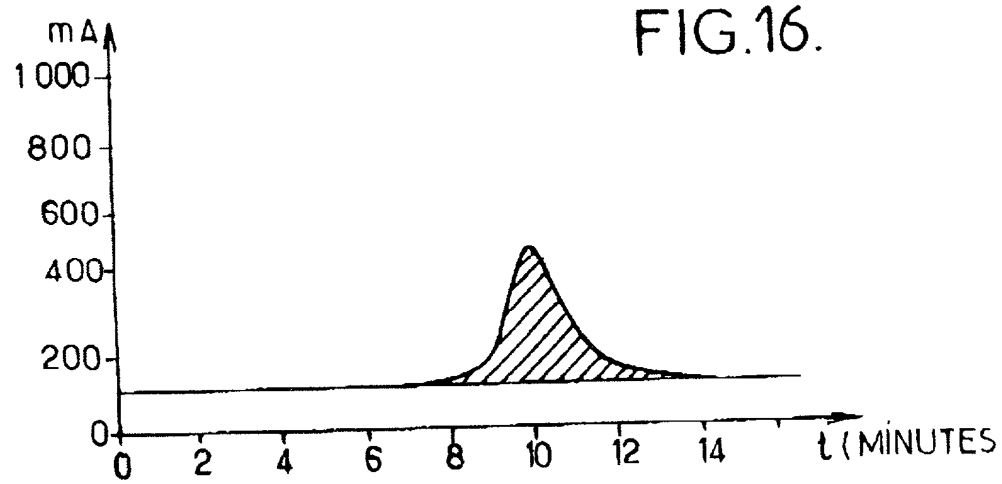
FIG.13.





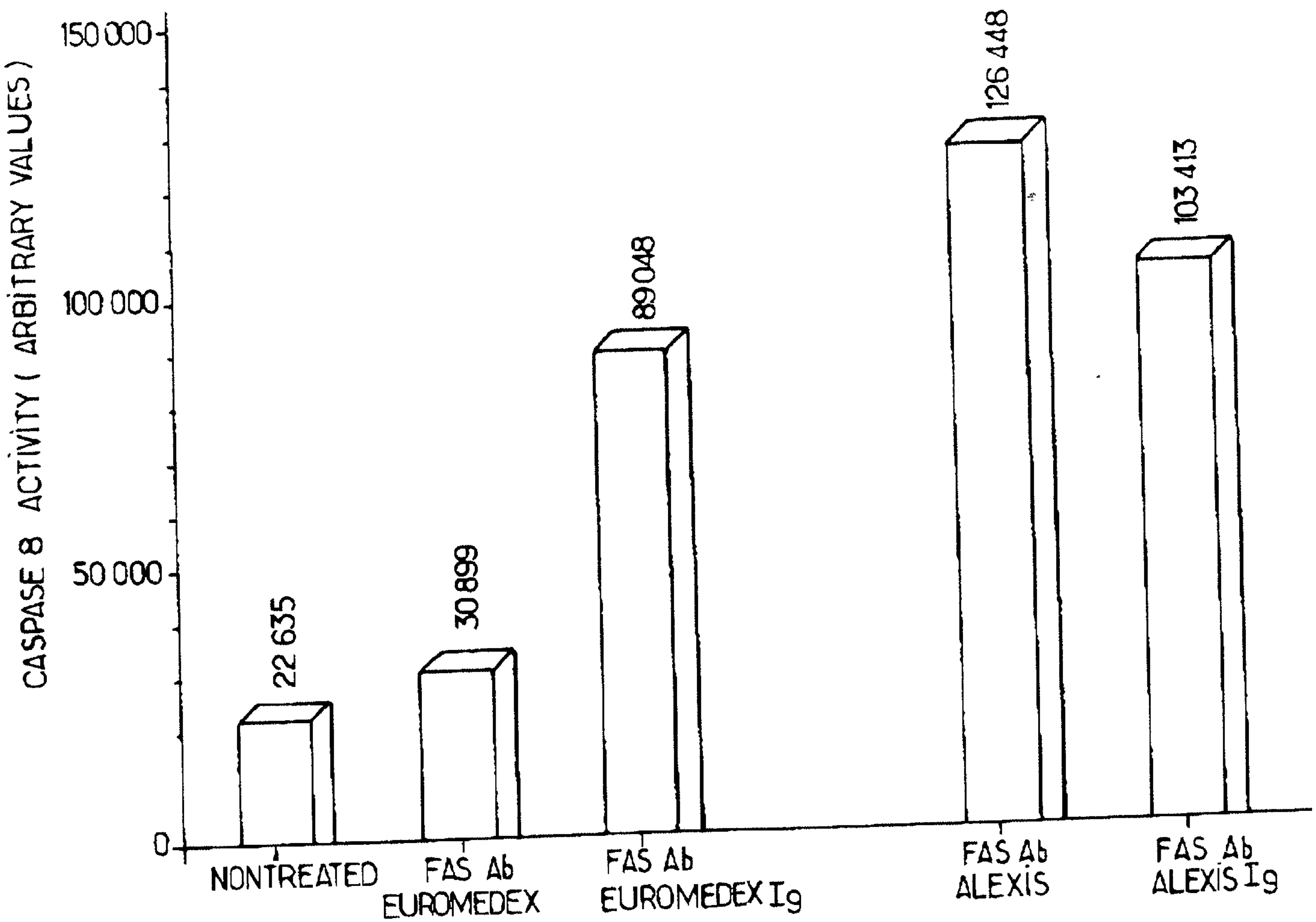
STIMULATION (%) FOR Ig + Fas AB
40
20
0
-20
-40
a
b
c
d
e
f
g
h
10
i
l
m
20
n
30
p
40
q
Fas Ab(%)
Ig
INHIBITION (%)
FOR Ig + Fas AB

FIG.14.



mA
1000
800
600
400
200
0
0
2
4
6
8
10
12
14
t (MINUTES)

FIG.16.



CASPASE 8 ACTIVITY (ARBITRARY VALUES)
150 000
100 000
50 000
0
22 635
30 899
89 048
126 448
103 413
NONTREATED
FAS Ab EUROMEDEX
FAS Ab EUROMEDEX Ig
FAS Ab ALEXIS
FAS Ab ALEXIS Ig

FIG.15.

DECLARATION AND POWER OF ATTORNEY Attorney's Docket No. 16721-0023

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name. I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: Medicine for treating apoptosis ...., the specification of which

☐ is attached hereto.

☒ was filed on August 3, 2000 as U.S. Application or PCT International Application No. 09/601,665
and was amended (if applicable) on ______________________.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above. I do not know and do not believe that the same was ever known or used by others in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to the date of this application. I further state that the invention was not in public use or on sale in the United States of America more than one year prior to the date of this application. *I understand that I have a duty of candor and good faith toward the Patent and Trademark Office,* and I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate disclosing subject matter in common with the above-identified specification and having a filing date before that of the application on which priority is claimed:

Country	App. No.	Date of Filing	Priority Claimed Under 35 USC §119
FRANCE	98 01237	03/02/1998	Yes X No ____

I hereby claim the benefit under Title 35, United States Code, § 120 of any prior United States application(s), or §365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each claim of the present application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations §1.56, which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Application No.	Filing Date	Status: patented, pending, abandoned
PCT/FR99/00229	03/02/1999	

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statement were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patents issuing thereon.

I hereby authorize the U.S. attorneys named herein to accept and follow instructions from **Cabinet Plasseraud**, as to any action to be taken in the Patent and Trademark Office regarding this application, without direct communication between the U.S. attorney and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney named herein will be notified by the undersigned.

35

POWER OF ATTORNEY: The following attorneys are hereby appointed to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: Anthony B. Askew - 24,154; Roger T. Frost - 22,176; Jeffrey E. Young - 28,490; Robert E. Richards - 29,105; John R. Harris - 30,388; Stephen M. Schaetzel - 31,418; Larry A. Roberts - 31,871; Thomas A. Hodge, - 22,602; Gregory T. Gronholm - 32,415; Dale Lischer - 28,438; Peter G. Pappas - 33,205; James Dean Johnson - 31,771; W. Scott Petty - 35,645; Daniel J. Warren - 34,272; Leona G. Young - 37,266; Jamie L. Greene-32,467; Holmes J. Hawkins III - 38,913; Mary Anthony Merchant - 39,771; Michael J. Mehrman - 40,086; William L. Warren - 36,714; Brenda Ozaki Holmes - 40,339; James D. Withers - 40,376; Kimberly J. Prior - 41,483; William B. Lafferty - 39,259; Theodore M. Green - 41,801; Christopher J. Leonard - 41,940; Christos S. Kyriakou - 42,776; John K. McDonald - 42,860; Michael S. Pavento - 42,985; Suzanne Seavello Shope - 37,933; M. Scott Boone - 42,341; Sima Singadia Kulkarni - 43,732; A. Shane Nichols - 43,836; Christopher J. Chan - 44,070; Collen A. Beard - 38,824

Send correspondence to: **JONES & ASKEW, LLP**
2400 Monarch Tower
3424 Peachtree Road, N.E.
Atlanta, GA 30326

Direct telephone calls **(404)949-2400** to:
Roger T. Frost

180

Full name of sole or first inventor:	Jean-Claude YVIN
Citizenship:	French
Residence:	3, rue Gabriel Desgrés, 35400 SAINT-MALO, FRANCE FRX
Post Office Address:	
Inventor's signature	Date: 22.11.2000

☒ Additional inventors are being named on separately numbered sheets attached hereto.

Attorney Docket No.: 16721-0023
Title: Medicine for Treating Apoptosis Dysfunctions Containing Oligosaccharides
Page 2

2e **Full name of second joint inventor, if any:**	Florence CRUZ		
Citizenship:	French		
Residence:	7 rue de la Loutre, 35400 SAINT-MALO, FRANCE FX		
Post Office Address:			
Inventor's signature		**Date:**	22.11.2000

3e **Full name of third joint inventor, if any:**	Valérie DESCAMPS		
Citizenship:	French		
Residence:	22 rue Yan d'Argent, 29680 ROSCOFF, FRANCE FX		
Post Office Address:			
Inventor's signature		**Date:**	22.11.2000

4e **Full name of fourth joint inventor, if any:**	Christophe RICHARD		
Citizenship:	French		
Residence:	Kernevez, 29400 PLOUGOURVEST, FRANCE FX		
Post Office Address:			
Inventor's signature		**Date:**	22.11.2000

5e **Full name of fifth joint inventor, if any:**	Vesna THIBAL		
Citizenship:	Croatian		
Residence:	5 rue Gentille, 69002 LYON, FRANCE FX		
Post Office Address:			
Inventor's signature		**Date:**	22.11.2000

6e **Full name of sixth joint inventor, if any:**	Patrick ARRIGO		
Citizenship:	French		
Residence:	Jussy, 74930, PERS-JUSSY, FRANCE FX		
Post Office Address:			
Inventor's signature		**Date:**	22.11.2000

7e **Full name of seventh joint inventor, if any:**	Bernard CLOAREC		
Citizenship:	French		
Residence:	81 rue de la Rive, 29250 SAINT POL DE LEON, FRANCE FX		
Post Office Address:			
Inventor's signature		**Date:**	22.11.2000